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6. AUTHOR(S) John J. Stegeman					
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13. ABSTRACT (Maximum 200 words) This grant examined cytochrome P450 induction in endothelium, a potentially critical yet largely unexplored site of xenobiotic action in vertebrates. The regulation and function of endothelial CYP1A, and its role in toxicity and pharmacokinetics of foreign compounds were examined in porcine and human aorta endothelial cells, and tissues from humans and non-mammalian vertebrates. The first endothelial dose-response to TCDD and first demonstration that endothelial cell CYP1A is catalytically active in intact cells were obtained. Response to different potency AhR agonists was determined. Studies of opportunity examined endothelial CYP1A induction as a marker of exposure, including in human placenta. The possibility that CYP1A in the endothelium might participate in toxicity of Ah receptor agonists was examined in several studies with fish models. The studies: provided new information important for evaluating both the ecological and health risk of chemicals; validated immunohistochemical analysis of CYP1A expression as a biomarker of environmental exposure to diverse compounds of interest to the Air Force; demonstrated endothelial cells as a novel, alternative system for evaluating new chemicals. Ten publications are appended.					
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Cytochrome P450 1A Induction in Vertebrate Endothelium

Final Report on AFOSR grant F40620-94-1039.

John J. Stegeman, Senior Scientist
Biology Department
Woods Hole Oceanographic Institution
Woods Hole, Massachusetts 02543

SUMMARY OF PROPOSAL AND RESULTS.

This grant was to examine cytochrome P450 induction in the endothelium, a potentially critical yet largely unexplored site of xenobiotic action in vertebrate organisms. The background to these studies includes the essential role of endothelium in the regulation of cardiovascular and other functions, and the earlier findings of very strong induction of CYP1A in endothelium. Though a single cell layer, the mass of endothelium throughout the vertebrate body constitutes a large organ, that could be an important site of toxic action, a major site of xenobiotic metabolism or removal, and a preferred site for biomarker analysis. Endothelium is a site of induction common to all organs. At the time of submission, CYP1A induction had been seen in endothelium of multiple species. Those findings implied that regulatory mechanisms for CYP1A genes are operating in endothelium, and that toxicity associated with receptor binding may occur there, i.e., that the endothelium itself could be a critical target for some chemical effects. Results also suggested the hypotheses that the endothelium may be an important site for xenobiotic removal from the circulatory system. This process could influence both the pharmacokinetics and the systemic toxicity of organic chemicals, particularly at low doses.

Studies to address these issues were proposed in two areas.

A. First we proposed to establish the utility of endothelial cells in culture for investigating the regulation and function of this cell type. 1. It was proposed to examine the sensitivity of endothelial cell response to model compounds, determining the dose-response for induction of cytochrome P450 1A in endothelial cells from mammalian and non-mammalian vertebrate species. 2. The effectiveness and magnitude of response for selected compounds that are weak and strong Ah-receptor agonists, and those which are rapidly or slowly metabolized were to be compared. 3. The rates of xenobiotic biotransformation in the endothelium were to be determined for selected substrates. 4. The sensitivity and magnitude of response, and the capacity for metabolism in endothelial cells were to be compared with those in hepatic cells in culture.

B. Secondly, studies were proposed to investigate the potential for endothelial CYP1A to act in toxicity and to influence pharmacokinetics of foreign compounds. Studies were designed to determine whether induction of cytochrome P450 1A in endothelium could affect the internal dose of toxicant at selected other target sites in the organism. The studies were to be carried out with mammalian and non-mammalian vertebrate species, to establish the common features of this response, providing a further basis for judging the validity of extrapolation between species. Although the studies proposed focused on cytochrome P450 1A, the *in vitro* systems developed could be useful to evaluate the role of other enzymes (e.g. CYP2E forms) in the effects and/or removal of unknown and novel toxicants.

It had been proposed to investigate metabolism of diverse chemicals in endothelial cells, as compared to hepatocytes, in collaboration with personnel at Air Force laboratories. However, due to a reduction in the period of award from 3 to 2 years, this could not be done.

RESULTS:

A. ENDOTHELIAL CELLS IN CULTURE:

Most of these objectives were accomplished. Studies were carried out with porcine and with human aorta endothelial cells. Responsiveness of endothelial cells in culture to induction of CYP1A and catalytic function of endothelial CYP1A were established. The first dose-response to TCDD, and several other inducers, and the first clear demonstration that endothelial cell CYP1A is catalytically active in intact cells were obtained. The response to different potency AhR agonists was determined. The temporal response to slowly metabolized (3,3',4,4'-tetrachlorobiphenyl) and rapidly metabolized (benzo[a]pyrene and β -naphthoflavone) inducers was determined. CYP1A activity with different substrates was determined. Comparison to hepatocytes is still being done.

In addition to the proposed studies, we were able to demonstrate that glucocorticoids act to potentiate the induction of CYP1A in endothelial cells.

Much of this progress has been published. (Appended)

Studies to compare mammalian and fish endothelial cells in culture were not accomplished as our efforts to establish endothelial cells from fish have not yet been successful.

B. ROLE OF ENDOTHELIAL CYP1A IN PHARMACOKINETICS AND TOXICITY:

Endothelial CYP1A and pharmacokinetics.

Studies proposed to examine the rete mirabile (a vascular bed) in the eel have been accomplished and demonstrate the catalytic function of fish endothelial CYP1A, and provide substantial support for the hypothesis that the "penetration" into distal parts of the animal can be strongly influenced by endothelial CYP1A.

This work has been presented and is being prepared for publication. (Not appended)

The possibility that CYP1A in endothelium might act as a "binding protein" as well as being able to metabolize compounds was examined with the fish scup CYP1A and 3,3',4,4'-tetrachlorobiphenyl. Immunoprecipitation results showed that the fish CYP1A1 can act as a binding protein in vivo and in vitro. This is novel in that in mammals, it is thought that CYP1A2 but not CYP1A1 acts as a binding protein. If true, that would preclude endothelial 1A from being a binding protein in mammals, as only 1A1 is expressed in extrahepatic organs in mammals.

This work is being prepared for publication. (Not appended)

Similar studies proposed with rabbit were not accomplished, in part due to the reduction in time of the grant. However, alternate studies have been accomplished with human placenta as an "endothelium-rich" structure. Cellular localization of CYP1A expression in placenta of smoking and non-smoking mothers has been examined.

This work has been presented and is being prepared for publication. (Appended)

Endothelial CYP1A induction and toxicity of Ah receptor agonists/ CYP1A substrates:

The possibility that CYP1A in the endothelium might participate in toxicity of Ah receptor

agonists was examined in several studies with fish. The results show a strong association between the degree and localization of endothelial induction and toxicity of TCDD. Several papers have been published or submitted describing these results. The studies included development of zebrafish as a model to test cardiac and endothelial dysfunction, and included other compounds of interest to the Air Force (lower mol. wt. aromatics such as benzene and toluene)

Short communications have been published. (Appended)

In addition, studies to address the possible mechanisms by which the CYP1A might be toxic were done. The results indicate that a likely avenue is by the generation of reactive oxygen species by the CYP1A when it binds slowly metabolized substrates such as 3,3',4,4'-tetrachlorobiphenyl or TCDD.

This work has been presented and is being prepared for publication. (Appended)

In addition, other endothelial enzymes have been examined, as a prelude to further studies of the mechanisms of toxicity. Thus, studies have been initiated on fish nitric oxide synthases. The first studies on NOS in liver and heart of fish were on enzymology. Studies on interaction of CYP1A and NOS, and on NO and superoxide in the endothelium are planned.

Initial characterization studies have been presented (appended)

Endothelial CYP1A as a biomarker

Several studies of opportunity were carried out related to the use of endothelial CYP1A induction as a marker of exposure. The study involving human placenta (see above) is one. Other studies continued with fish, comparing the dose-responsiveness of induction in different cell types, as compared to the results in endothelium. The appearance of PAH-inducible P450s in endothelium of different organs of diverse vertebrates continues to indicate that this is a fundamental response in this cell type.

These studies have been presented and published or are being prepared for publication.

In summary, the studies succeeded in: 1. Providing new information important for evaluating both the ecological and health risk of chemicals. 2. Validating immunohistochemical analysis of CYP1A expression as a molecular biomarker for environmental exposure to diverse compounds including those of interest to the Air Force. 3. The studies demonstrated endothelial cells as a novel, alternative system for evaluating new chemicals.

Publications appended:

- 1995 Stegeman, J. J., M. E. Hahn, R. Weisbrod, B. R. Woodin, J. Joy, S. Najibi and R. Cohen. Induction of Cytochrome P450 1A1 by aryl hydrocarbon-receptor agonists in porcine aorta endothelial cells in culture, and CYP1A1 activity in intact cells. *Molecular Pharmacology*. **47**: 296-306.
- 1996 Mizell, M., E. Romig, J. J. Stegeman, R. M. Smolowitz and R. Katayani. Zebrafish embryo monitoring of the aquatic environment: Dose response synergism revealed in combination of pollutant chemical mixtures. *Biological Bulletin*. **191**: 29292-294

- 1996 Mizell, M., J. J. Stegeman, E. Romig, R. M. Smolowitz, J. J. Schlezinger, R. Katayani, B. R. Woodin and M. Mortensen. Chemically-induced cardiovascular defects in developmental stages of vertebrates: Dose-response and phenotypic comparisons in medaka and zebrafish exposed to aryl hydrocarbon receptor agonists. *Biological Bulletin*. **191**: 294-295
- 1996 Cox, R. and J. J. Stegeman. Characterization of nitric oxide synthase (NOS) in fish liver: Enzyme activity and immunoblot analysis. in: *Biology of Nitric Oxide*, Moncada, et al., eds. Portland Press, p. 47.
- 1997 Woodin, B. R., R. M. Smolowitz and J. J. Stegeman. Induction of cytochrome P450 1A in the intertidal fish *A. purpureus* by Prudhoe Bay crude oil and environmental induction in fish from Prince William Sound. *Environ. Science and Technol.* **31**: 1198-1205
- 1997 Guiney, P. D., R. M. Smolowitz, R. E. Peterson and J. J. Stegeman. Correlation of 2,3,7,8-Tetrachlorodibenzo-p-dioxin induction of cytochrome P450 1A in vascular endothelium with toxicity in early life stages of lake trout. *Toxicol. Appl. Pharmacol.* **143**: 256-273.
- 1997 Celander, M., R. Weisbrod and J. J. Stegeman. Glucocorticoid potentiation of cytochrome P450 1A1 induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin in porcine and human endothelial cells in culture. *Biochem. Biophys Res. Comm.* **232**: 749-753.
- 1997 Schlezinger, J. R. D. White and J. J. Stegeman. 3,3',4,4'-Tetrachlorobiphenyl Stimulates Release of Active Oxygen from Hepatic Microsomes and the Oxidative Inactivation of Cytochrome P450 1A1. (Presented at SOT, 1997). *The Toxicologist* **36**: 692.
- 1997 Deng, Y. F., B. P. Slezak, A. T. Draushuk, J. J. Stegeman and J. R. Olson. Induction of cytochrome P450 1A1 in human placenta with cigarette smoking: effects of Exon 7 and MSP1 polymorphism. *The Toxicologist* **36**: 22.
- 1997 Slezak, B. D., J. Fisher, M. Alashari, J. Schlezinger, J. J. Stegeman and J. R. Olson. Regional and cell-specific expression of cytochrome P450 1A1 in human placenta. *The Toxicologist* **36**: 22.